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Abstract: PURPOSE To examine if dietary intake of foods rich in flavonoids, which have been shown to be inversely associated with chronic diseases, is associated with inflammatory processes. METHODS This analysis includes controls of case-control studies nested within the Multiethnic Cohort (MEC) who completed a validated food frequency questionnaire at cohort entry. Biomarkers were assessed in blood donated during follow-up (mean = 9.6 years). We used multivariate linear regression adjusted for potential confounders to estimate associations between intake of flavanones, flavonols, and isoflavones and levels of adiponectin, leptin, C-reactive protein, interleukin (IL)-1 , IL-6, IL-10, and tumor necrosis factor- . RESULTS Among the 1,287 participants, the respective median intakes of flavanones, flavonols, and isoflavones were 26.5, 12.4, and 1.3 mg/day at cohort entry. With the exception of flavanone intake, which was statistically significantly inversely associated with adiponectin ($p = 0.01$) and IL-6 concentrations ($p = 0.01$), none of the examined flavonoids was related with levels of adipokines or inflammatory markers. Heterogeneity by ethnicity was only observed for flavonol intake and IL-10 ($p = 0.04$) and may be the result of multiple testing. These null findings were confirmed in a subset of participants who completed a second dietary history within 2.6 years of blood draw. CONCLUSION The current results do not support a consistent association between dietary intake of flavonoids and markers of inflammatory processes.

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Self-reported dietary flavonoid intake and serum markers of inflammation: The Multiethnic Cohort

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Abstract

Purpose: To examine if dietary intake of foods rich in flavonoids, which have been shown to be inversely associated with chronic diseases, is associated with inflammatory processes.

Methods: This analysis includes controls of case-control studies nested within the Multiethnic Cohort (MEC) who completed a validated food frequency questionnaire at cohort entry. Biomarkers were assessed in blood donated during follow-up (mean=9.6 years). We used multivariate linear regression adjusted for potential confounders to estimate associations between intake of flavanones, flavonols, and isoflavones and levels of adiponectin, leptin, C-reactive protein, interleukin (IL)-1β, IL-6, IL-10 and tumor necrosis factor-α.

Results: Among the 1,287 participants, the respective median intakes of flavanones, flavonols, and isoflavones were 26.5, 12.4, and 1.3 mg/day at cohort entry. With the exception of flavanone intake, which was statistically significantly inversely associated with adiponectin ($p=0.01$) and IL-6 concentrations ($p=0.01$), none of the examined flavonoids was related with levels of adipokines or inflammatory markers. Heterogeneity by ethnicity was only observed for flavonol intake and IL-10 ($p_{interaction}=0.04$) and may be the result of multiple testing. These null findings were confirmed in a subset of participants who completed a second dietary history within 2.6 years of blood draw.

Conclusion: The current results do not support a consistent association between dietary intake of flavonoids and markers of inflammatory processes.

Keywords: flavonoids, isoflavones, lignans, inflammation, leptin, adiponectin

Introduction

Inflammatory processes are involved in cancer development and progression [1, 2]. Thus, factors that influence these processes, such as nutrients with antioxidant capacity, may potentially influence cancer risk. Polyphenols are antioxidants found in abundance in fruits, vegetables, cereals, dry legumes, chocolate, and beverages, such as tea, coffee, or wine [3]. Flavonoids, a subgroup of polyphenols, are classified into the main sub-groups of flavonols, flavones, flavanols, flavanones, isoflavones, lignans, anthocyanins and proanthocyanins [4]. Whereas experimental studies on animals or cultured human cell lines support a role of flavonoids in the prevention of cancers, the associations in human studies are less clear, although some studies support their association with the risk of certain types of cancer [5, 6]. For example, in a lung cancer case-control study conducted in Hawaii, foods rich in quercetin (onions and apples) and naringenin (white grapefruit) were inversely associated with risk for certain forms of lung cancer [7]. In the Multiethnic Cohort (MEC), total flavonol intake, and in particular, kaempferol, was inversely related to pancreatic cancer [8], and total intake of isoflavones, as well as the intake of daidzein and genistein individually, were related to a lower endometrial cancer risk [9]. Although it has been speculated that flavonoids might influence cancer risk via effects on inflammation [10], so far only a few investigations have examined the associations of flavonoid intake with markers of inflammation. The results are rather heterogeneous depending on the flavonoids and the inflammatory markers examined, as well as on the type of study [11-19]. Almost all studies that analyzed the associations between flavonoids and inflammation have been conducted in white populations; only studies based on the National Health and Nutrition Examination Survey (NHANES) included non-Hispanic Blacks and Mexican-Americans but without reporting results by race/ethnicity [16, 17]. The MEC offers a good opportunity to investigate whether associations between intake of flavonoids and inflammatory markers in blood differ across ethnic groups, in particular in whites and Asians given their diverse dietary habits and patterns of flavonoid intake. Specifically, we examined the intake of isoflavones, found mainly in soy foods, flavonols (kaempferol, quercetin, myricetin, commonly found in onions and apples), and flavanones (naringenin and hesperitin as found in citrus fruits), which were currently available in our food composition database.

Material and methods

Study participants. The MEC is a longitudinal study designed to investigate the association of dietary, lifestyle, and genetic factors with the incidence of cancer. The cohort was established in Hawaii and Los Angeles in 1993-1996 [20]. Individuals from 5 major ethnic groups (whites, Japanese Americans, Latinos, African Americans, and Native Hawaiians) were identified primarily through driver's license files, but also through voter registration lists in Hawaii and Medicare files in California. A total of 215,251 men and women aged 45 to 75 years were included at baseline [20]. All participants completed a self-administered questionnaire on diet, anthropometric measures, medical history, and lifestyle. A MEC biospecimen subcohort was established in 2001-2006 by asking surviving cohort members to provide blood and urine specimens [21]. In total, 67,594 cohort members, broadly representative of the full cohort, contributed to the biorepository. During 2003-2007, a full questionnaire (Qx3) was completed by approximately 50%of cohort members. The study protocol was approved by the Institutional Review Boards of the University of Hawaii and the University of Southern California.

Dietary assessment. A validated food frequency questionnaire (FFQ) was used to assess the participants' habitual diet in the past year [22]. For selected macro- and micronutrients, the correlations with 24-hour recalls were highly satisfactory after energy adjustment, but individual foods and nutrients such as flavonoids were not validated separately [22]. Based on FFQ information, daily intake of selected flavonoids and total energy intake were computed using a food composition table developed and maintained by the University of Hawaii Cancer Center [23]. It includes primarily information from the U.S. Department of Agriculture but also measurements performed at our institution. For a subset of study participants, updated diet information was collected by FFQ as part of Qx3. Flavonoids that were available in the nutritional database included flavonols (kaempferol, quercetin, myricetin), flavanones (naringenin glycosides, hesperitin glycosides), and isoflavones (genistein, dadzein, glycitein), all measured as glycosides [24, 25]. Other flavonoids were not available at this time and therefore not included in the current analysis. To assess diet quality, the Health Eating Index 2010 was computed for study participants as described previously [26].

Laboratory assays. To evaluate the association between polyphenol intake and serum concentrations of selected adipokines and inflammatory markers, we included 1287 controls

from nested case-control studies on non-Hodgkin lymphoma [27] and breast cancer [28]. Several adipokines and inflammatory markers were measured in these studies, including pro-inflammatory cytokines (interleukin [IL]-1 β, IL-6, IL-10, and tumor necrosis factor [TNF]-α), C-reactive protein (CRP), leptin, and adiponectin. All assays were performed by the Analytical Biochemistry Shared Resource at the University of Hawaii Cancer Center following the manufacturer's protocol unless noted otherwise [27, 28]. Frozen serum samples were retrieved from the MEC biorepository and analyzed in duplicate to quantify leptin and adiponectin using a double-antibody enzyme-linked-immunosorbent-assay (R&D Systems, Minneapolis, MN, U.S.A.). CRP was assessed using a Cobas MiraPlus clinical chemistry analyzer (Roche Diagnostics, Indianapolis, IN) and a latex particle enhanced immunoturbidimetry-based kit from Pointe Scientific (Lincoln Park, MI). TNF-α, IL-1β, IL-6, and IL-10 were included in a Luminex panel, measured using a modification of an Invitrogen (Carlsbad, CA) magnetic high sensitivity 10-plex assay (LHC0001), and quantified on a Luminex 200 plate reader. Intra-batch coefficients of variation based on 96 blinded duplicate and 9 triplet samples for leptin, adiponectin, CRP, TNF-α, IL-1β, IL-6 and IL-10 were 3.4-6.4%, 2.5-9.4%, 3.5-5.0%, 10.0%, 20.9%, 8.9%, and 7.8%respectively [27, 28].

Statistical analysis. Since dietary intake variables and serum biomarkers were not normally distributed, these variables were log-transformed to satisfy model assumptions. We used linear regression analysis to compute covariate-adjusted geometric mean concentrations of inflammatory markers and adipokines by tertiles of flavonoid (flavanones, flavonols, and isoflavones) intake. To evaluate trends, we modeled the flavonoids as log-transformed continuous variables. In all models, we took into account age at blood draw, sex, ethnicity, study indicator (breast or NHL), season of blood draw (October-March or April-September), total energy intake (log-transformed, continuous), body mass index (BMI, kg/ m^2, continuous, log-transformed), smoking status (never, past, current), physical activity (< vs. ≥1/2 hour moderate/vigorous activity per day), alcohol intake (<1 drink/month, ≥1/month and <1/day, ≥1/day), and the Healthy Eating Index 2010 [26]. The models were also tested for interaction of flavonoids with ethnicity. Ethnic-specific analyses were conducted when a significant interaction term was detected. For 957 cohort members with Qx3 data, the overall models were repeated to examine if diet closer to blood draw results in different findings. All analyses were performed with SAS software, version 9.3 (SAS Institute Inc., Cary, North Carolina).

Results

Of 1,287 study participants, 960 (74.6%) were female and 327 (25.4%) were male. The mean age at blood collection was 68.9 years, with men being slightly older than women. Approximately 25%of the participants were white, 32%Japanese American, 20%Latino, 16% African American, and 8%Native Hawaiian (Table 1). Mean time from cohort entry to blood draw was 9.6 years. Almost 50%of blood samples were collected during winter months. The prevalence of smoking at cohort entry was generally low, but differed among ethnic groups with the highest prevalence among African Americans (15.5%) and the lowest in Japanese Americans (6.3%). Median intakes of flavanones, flavonols, and isoflavones were 26.5, 12.4, and 1.3 mg/day, respectively. Intake of flavonols was similar across ethnic groups, whereas isoflavones and flavanone intake varied widely. Serum concentrations of inflammatory markers and adipokines also differed considerably across ethnic groups, with generally highest concentrations of inflammatory markers in African Americans and the lowest in Japanese Americans; the same was true for leptin levels.

None of the flavonoids available for analysis was statistically significantly associated with serum concentrations of adipokines or inflammatory markers (Table 2) with the exception of flavanone intake, which was statistically significantly inversely associated with adiponectin ($p=0.01$) and IL-6 concentrations ($p=0.04$). When we examined the relation of flavonoids with adipokines and inflammatory markers by ethnic group, we generally observed no significant associations with the exception of statistically significant effect modification of the association between flavonol intake and IL-10 concentration ($p_{interaction}= 0.04$), but the association between flavonol intake and IL-10 concentration was not statistically significant in any racial/ethnic group after stratification (data not shown).

The mean time between Qx3 and blood draw was 2.0±2.6 years for the 957 participants who completed Qx3; of these 861 donated blood before and 114 after Qx3. Median intakes at Qx3 were estimated as 17.0 (Q1, Q3: 5.2, 45.9) mg for flavanones, 12.9 (7.6, 19.6) mg for flavonols, and 3.3 (0.9, 8.8) mg for isoflavones. The values at cohort entry and Qx3 were significantly correlated ($p<0.0001$ for all): flavonols ($r_s=0.49$), flavanones ($r_s=0.43$), and isoflavones ($r_s=0.58$) despite the differences in medians. Diet quality as indicated by the HEI-2010 increased slightly from 69.8 to 74.4 from Qx1 to Qx3 and the correlation was comparable to the flavonoids ($r_s=0.49$; $p<0.0001$). Using Qx3 data, the associations in the overall models were null for all models, even the p-values for trend of flavanones with adiponectin and IL-6 concentrations were non-significant ($p=0.12$ and 0.22,

respectively). Geometric mean concentrations by tertiles of flavanone intake were 7.9, 8.0, and 8.5 μg/mL for adiponectin and 3.3, 3.2 and 3.1 pg/mL for IL-6 (data not shown).

Discussion

In this multiethnic US cohort, dietary intake of different classes of flavonoids, i.e., flavanones, flavonols and isoflavones, measured at two points in time was generally not associated with circulating concentrations of adipokines and inflammatory markers. Very few statistically significant associations were detected, but there was no systematic pattern. Therefore, the multiple comparisons probably led to a few chance findings. Using dietary intake data closer to the blood draw (2 instead of 9 years) did not change the results substantially.

Although flavonoids have been shown to be associated with inflammatory processes in vitro and in vivo [10], only a few epidemiological studies examined the associations of flavonoid intake with inflammation and reported significant associations in contrast to the current findings. In NHANES, intake of flavonols was inversely associated with CRP concentration, but there was no association with intake of flavanones [11]. In a UK study, intake of flavanones and flavonols was not associated with serum concentrations of adiponectin and CRP in a group of women aged 18-76 years [12]. In the Nurses' Health Study, intake of flavanones was related to lower IL-18 concentration and intake of flavonols with lower concentration of soluble vascular cell adhesion molecule-1, but neither flavanone intake nor flavonol intake was associated with concentrations of CRP, soluble tumor-necrosis factor-2 (sTNF-2), or IL-6 [13]. In the Women's Health Study, intake of flavonols was not associated with plasma concentrations of CRP or IL-6 [14].

Our results on soy isoflavones that may act as weak estrogens, antioxidants and/or anti-inflammatory agents [29] also disagree with published evidence. Dietary isoflavone intake was inversely associated with concentration of CRP in two reports [15, 30]; in NHANES, higher urinary excretion of isoflavones was related to lower CRP concentration and lower white blood cell count [16]. However, the results of intervention trials with isoflavones (as reviewed in Ref.[16]) and lignans [18, 19] were heterogeneous. In contrast to isoflavones, which are more common in Asian diets, lignans, another group of phytoestrogens, are predominantly part of Western diets. Only one study, also conducted in NHANES, examined the association of lignan excretion with inflammatory markers and described an inverse association with concentrations of CRP and white blood cell count [17].

Strengths of this study include its multiethnic population and the population-based design. This allows for generalization of our results to non-white populations. Some

limitations need to be taken into account. First, blood samples were taken approximately 9 years after cohort entry, but the associations remained null in a subset of participants who provided an updated diet history within 2 years of the blood draw, however, mostly after the blood draw. As indicated by the correlations of the flavonoid values and the diet quality indicator (Hei-2010) between Qx1 and Qx3, dietary intake remained fairly stable over time, an observation that agrees with reports that dietary habits in adults usually do not change much, even over longer periods of time [31]. Second, we did not have data on the intake of lignans and other flavonoids available in our database. Third, isoflavones are metabolized into more active components by gut microbiota, the patterns of which differ across individuals [32]. For this reason and because circulating concentrations are independent of measurement errors due to imprecise dietary assessment and databases [33], measuring circulating concentrations of isoflavones and other flavonoids as biomarkers might provide a better assessment of active components than estimating dietary intake [34, 35]. However, it has been shown that FFQs provide a reasonable estimate of true biological exposure [36, 37]. Other weaknesses include the lack of a better measure for visceral fat than BMI, e.g., waist-hip ratio, which was not available. It is worthwhile to conduct similar analyses as our but with measured intake and circulating concentrations of flavonoids in order to (a) examine the association between intake and metabolism and (b) address the effect on inflammation.

In conclusion, our FFQ-based results do not indicate that the habitual dietary intake of certain groups of flavonoids, including isoflavones, affects circulating concentrations of adipokines and cytokines in this population-based cohort of older adults with different ethnic backgrounds. Due to the possible role of the gut microbiome in polyphenol metabolism [32], very short-term exposure may be the most relevant to answer this question, In that case, possible associations need to be examined when the time period between diet history and blood draw is much shorter than in the current investigation.

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Table 1. Characteristics of study participants by ethnic group[1]

Characteristic	All	African American	Native Hawaiian	Japanese American	Latino	White
Number of participants, %by ethnicity	1,287	15.5	7.9	31.9	20.1	24.7
Female participants, %	74.6	72.5	82.2	78.3	76.7	67.0
Current smoking at cohort entry[2]	9.5	15.5	10.9	6.3	7.4	11.0
Diabetes at cohort entry, %	6.8	7.5	7.9	7.8	11.2	1.3
Age at blood draw, years	68.9 (62.7, 74.5)	69.6 (65.0, 75.0)	65.8 (62.0, 72.0)	69.5 (61.7, 74.9)	68.2 (63.2, 73.3)	69.0 (62.0, 76.0)
Time cohort entry to blood draw, years	9.6 (8.2, 10.8)	9.4 (6.5, 11.0)	9.2 (7.1, 10.5)	9.7 (8.7, 10.7)	9.3 (7.3, 10.2)	10.1 (8.7, 11.5)
BMI at cohort entry, kg/m^2	25.4 (22.6, 28.7)	27.5 (24.2, 31.1)	27.3 (24.9, 30.6)	23.5 (21.5, 26.2)	27.5 (24.3, 30.1)	24.5 (22.4, 27.8)
Fasting hours before blood draw[2]	13.0 (11.3, 14.0)	13.3 (10.2, 15.0)	12.8 (11.6, 14.0)	13.0 (11.5, 14.0)	13.0 (11.0, 14.5)	12.8 (11.7, 13.6)
Blood collected October-March, %	46.5	51.5	47.5	47.6	42.3	45.0
Serum biomarker concentration						
Leptin, ng/mL[2]	14.9 (7.6, 28.5)	26.4 (10.3, 49.4)	20.2 (12.3, 35.1)	10.5 (5.8, 17.9)	18.6 (10.9, 36.2)	14.3 (6.4, 28.6)
Adiponectin, µg/mL	8.9 (5.3, 14.7)	7.5 (3.9, 12.9)	7.4 (5.1, 11.7)	7.7 (4.7, 13.9)	9.9 (6.4, 15.2)	10.8 (6.5, 18.8)
C-reactive protein, mg/L[2]	1.8 (0.8, 4.0)	3.3 (1.6, 6.8)	2.2 (0.8, 3.7)	1.0 (0.4, 2.1)	2.5 (1.4, 4.9)	2.0 (1.0, 4.0)
TNF-α, pg/mL	6.7 (2.5, 14.0)	7.6 (2.3, 14.6)	8.2 (2.9, 16.3)	6.5 (3.0, 12.9)	7.2 (2.0, 15.4)	6.0 (2.2, 12.5)
Interleukin-1β, pg/mL	1.3 (0.7, 2.3)	1.3 (0.7, 2.8)	1.2 (0.6, 2.0)	1.3 (0.8, 2.2)	1.4 (0.7, 2.6)	1.2 (0.6, 2.1)
Interleukin-6, pg/mL	2.8 (1.3, 5.5)	3.3 (1.6, 7.4)	3.4 (1.9, 5.6)	2.5 (1.2, 4.7)	3.1 (1.5, 5.4)	2.5 (1.2, 5.1)
Interleukin-10, pg/mL	1.8 (0.8, 3.6)	1.7 (0.9, 3.9)	2.3 (1.1, 3.5)	1.9 (0.9, 3.7)	1.7 (0.8, 3.5)	1.5 (0.7, 3.7)
Daily dietary intake at cohort entry						
Isoflavones, mg	1.3 (0.6, 3.5)	0.5 (0.2, 0.9)	2.9 (1.5, 5.6)	3.9 (2.5, 8.0)	0.9 (0.5, 1.4)	0.8 (0.4, 1.6)
Genistein, mg	0.7 (0.3, 1.7)	0.2 (0.1, 0.4)	1.4 (0.7, 2.7)	1.9 (1.2, 3.8)	0.4 (0.2, 0.7)	0.4 (0.2, 0.8)
Daidzein, mg	0.6 (0.2, 1.4)	0.2 (0.1, 0.4)	1.2 (0.6, 2.3)	1.6 (1.0, 3.2)	0.4 (0.2, 0.6)	0.3 (0.2, 0.7)
Glycitein, mg	0.1 (0.1, 0.4)	0.0 (0.0, 0.1)	0.3 (0.2, 0.7)	0.4 (0.3, 0.9)	0.1 (0.0, 0.1)	0.1 (0.0, 0.2)
Flavonols, mg	12.4 (7.9, 18.7)	11.0 (7.2, 16.7)	12.4 (7.7, 18.3)	13.3 (8.2, 19.6)	12.6 (8.3, 20.2)	11.7 (7.5, 18.6)
Kaempferol, mg	2.9 (1.7, 5.0)	3.0 (1.7, 5.1)	3.3 (2.1, 5.1)	3.5 (2.2, 5.6)	2.3 (1.3, 4.0)	2.5 (1.6, 4.8)
Myricetin, mg	0.5 (0.3, 1.2)	0.4 (0.2, 0.7)	0.5 (0.2, 1.0)	0.6 (0.3, 1.3)	0.5 (0.2, 0.9)	0.6 (0.3, 1.4)
Quercetin, mg	8.8 (5.5, 12.6)	7.1 (5.0, 11.3)	8.6 (5.2, 11.6)	8.9 (5.6, 12.4)	9.9 (6.1, 14.4)	8.6 (5.6, 12.7)
Flavanones, mg	26.5 (8.3, 63.6)	36.1 (11.0, 80.0)	18.1 (7.1, 55.5)	23.8 (7.1, 52.7)	33.2 (11.7, 82.1)	20.7 (7.5, 56.6)
Naringenin glycosides, mg	2.3 (0.5, 6.0)	3.3 (0.7, 6.7)	1.3 (0.2, 6.4)	1.8 (0.5, 5.6)	2.4 (0.7, 6.5)	2.3 (0.4, 6.2)
Hesperitin glycosides, mg	22.3 (7.0, 55.9)	31.7 (9.6, 72.8)	17.9 (6.1, 49.1)	19.2 (5.8, 47.8)	29.8 (10.2, 73.4)	17.7 (5.9, 46.2)
Total energy, kcal/day	1827 (1380, 2443)	1654 (1230, 2420)	2037 (1358, 2504)	1798 (1418, 2385)	1926 (1354, 2789)	1825 (1409, 2348)
Vegetables, g/day	296.3 (193.0, 426.0)	241.6 (167.7, 383.3)	336.0 (171.8, 452.3)	298.1 (198.6, 425.1)	319.3 (202.1, 483.1)	285.1 (198.5, 415.9)
Fruits and fruit juice, g/day	291.7 (154.0, 477.9)	285.6 (145.6, 509.6)	263.0 (115.8, 518.7)	286.9 (148.9, 453.1)	318.2 (176.7, 544.2)	293.5 (170.6, 462.5)
Dietary fiber, g/day	21.7 (15.1, 30.3)	21.0 (14.6, 29.9)	21.6 (12.4, 29.3)	20.3 (14.5, 26.7)	27.0 (16.5, 40.0)	22.0 (15.8, 29.7)
Alcohol, g/day	0.0 (0.0, 3.7)	0.0 (0.0, 3.5)	0.0 (0.0, 4.0)	0.0 (0.0, 0.4)	0.0 (0.0, 2.2)	3.1 (0.0, 16.7)

[1] Data are given as median (interquartile range) or n (%); percentages may not add to 100 due to rounding.

[2] Data are missing for fasting hours prior to blood draw (N=18), current smoking at cohort entry (N=16), leptin (N=1), and C-reactive protein (N=6).

Table 2. Serum concentrations of biomarkers[1,2] by tertiles of dietary flavonoid intake at cohort entry, Multiethnic Cohort

Analyte[3]	Leptin [ng/mL]		Adiponectin [µg/mL]		CRP [mg/mL]		TNF-α [pg/mL]		IL-6 [pg/mL]		IL-1ß [pg/mL]		IL-10 [pg/mL]	
	Geometric mean	95%CI	Geometric mean	95%CI	Geometric mean	2.195%CI	Geometric mean	95%CI	Geometric mean	95%CI	Geometric mean	95%CI	Geometric mean	95%CI
Flavanones														
T1 (4.7 mg/d)	12.8	(11.0, 14.9)	7.5	(6.3, 8.7)	2.1	(1.6, 2.6)	6.5	(4.8, 8.8)	2.8	(2.1, 3.8)	1.3	(1.0, 1.7)	2.2	(1.5, 3.1)
T2 (26.5 mg/d)	12.3	(10.5, 14.3)	7.4	(6.3, 8.7)	1.8	(1.4, 2.2)	6.1	(4.4, 8.3)	2.6	(1.9, 3.5)	1.3	(1.0, 1.7)	2.0	(1.3, 2.8)
T3 (86.3 mg/d)	12.0	(10.3, 14.0)	8.4	(7.2, 9.8)	2.0	(1.6, 2.5)	6.5	(4.8, 8.9)	3.1	(2.1, 4.1)	1.3	(1.0, 1.7)	2.1	(1.4, 3.0)
p-trend[4]	*0.51*		*0.01*		*0.33*		*0.32*		*0.04*		*0.30*		*0.35*	
Flavonols														
T1 (6.6 mg/d)	12.4	(10.6, 14.4)	8.2	(7.0, 9.6)	2.0	(1.6, 2.5)	6.4	(4.7, 8.7)	2.9	(2.1, 3.9)	1.4	(1.0, 1.8)	2.0	(1.4, 2.9)
T2 (12.4 mg/d)	12.7	(10.9, 14.8)	7.6	(6.5, 9.0)	1.9	(1.5, 2.4)	6.1	(4.4, 8.3)	2.7	(1.9, 3.6)	1.3	(0.9, 1.7)	2.0	(1.3, 2.8)
T3 (22.1 mg/d)	12.0	(10.3, 14.0)	7.6	(6.5, 8.9)	2.0	(1.5, 2.4)	6.5	(4.8, 8.8)	2.9	(2.1, 3.9)	1.3	(1.0, 1.7)	2.2	(1.5, 3.1)
p-trend[4]	*0.19*		*0.84*		*0.67*		*0.24*		*0.31*		*0.59*		*0.27*	
Isoflavones														
T1 (0.4 mg/d)	12.6	(10.8, 14.6)	7.9	(6.7, 9.2)	1.9	(1.5, 2.4)	6.3	(4.6, 8.5)	2.9	(2.1, 3.9)	1.3	(1.0, 1.7)	2.2	(1.5, 3.0)
T2 (1.3 mg/d)	12.4	(10.6, 14.6)	7.8	(6.6, 9.3)	2.1	(1.7, 2.7)	5.9	(4.2, 8.1)	2.6	(1.9, 3.6)	1.3	(1.0, 1.8)	2.2	(1.5, 3.1)
T3 (4.9 mg/d)	12.0	(10.2, 14.0)	7.6	(6.5, 9.0)	1.8	(1.4, 2.3)	6.8	(5.0, 9.3)	2.9	(2.1, 4.0)	1.3	(0.9, 1.7)	2.0	(1.3, 2.8)
p-trend[4]	*0.57*		*0.79*		*0.05*		*0.53*		*0.45*		*0.88*		*0.78*	

[1] Values are estimates from analysis of covariance of serum biomarkers; all serum biomarkers and dietary polyphenol intake were log-transformed. Data are missing for serum leptin (N=1), C-reactive protein (N=6).

[2] Geometric means were adjusted for age, sex, ethnicity, study, time of blood draw (October-March or April-September), total energy intake (log-transformed), BMI (log-transformed), Healthy Eating Index-2010 (continuous), alcohol intake, physical activity, and smoking.

[3] Median intake (mg/day) within each tertile category (T1-T3) is presented in parenthesis.

[4] Trend test is based on log-transformed continuous flavonoid variable.